Developmental Genetic Analysis of *Contrabithorax* Mutations in *Drosophila melanogaster*

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ABSTRACT

A developmental analysis of the *Contrabithorax* (*Cbx*) alleles offers the opportunity to examine the role of the *Ultrabithorax* (*Ubx*) gene in controlling haltere, as alternative to wing, morphogenesis in Drosophila. Several *Cbx* alleles are known with different spatial specificity in their wing toward haltere homeotic transformation. The molecular data on these mutations, however, does not readily explain differences among mutant phenotypes. In this work, we have analyzed the "apogenetic" mosaic spots of transformation in their adult phenotype, in mitotic recombination clones and in the spatial distribution of Ubx proteins in imaginal discs. The results suggest that the phenotypes emerge from early clonality in some *Cbx* alleles, and from cell-cell interactions leading to recruitment of cells to *Ubx* gene expression in others. We have found, in addition, mutual interactions between haltere and wing territories in pattern and dorsoventral symmetries, suggesting short distance influences, "accommodation," during cell proliferation of the anlage. These findings are considered in an attempt to explain allele specificity in molecular and developmental terms.

NULL alleles of genes with morphogenetic phenotypes usually display complete penetrance and expressivity but spatial specificity (TIMOFEEFF-RESSOVSKI 1937). This last property is used to define the realm of action of the gene. However, leaky alleles of the same genes may show incomplete penetrance, variable expressivity and variable spatial specificity within the region affected by the total lack-of-function alleles (GARCÍA-BELLIDO and CAPDEVILA 1978). This is the case for alleles in the *Ultrabithorax* gene of *D. melanogaster*. Phenotypic analyses of null alleles of this gene led LEWIS (1963, 1978; see also MORATA and GARCÍA-BELLIDO 1976; KERRIDGE and MORATA 1982; MIÑANA and GARCÍA-BELLIDO 1982) to define its realm of action as limited to particular segments and compartments (from posterior mesothorax, T2p to anterior first abdominal, A1a). the availability of molecular probes for this gene [for RNA *in situ* hybridization, see AKAM (1983) and of antibodies against Ubx proteins (UBX) WHITE and WILCOX (1985a)] has allowed visualization and thus confirmation of the proposed spatial pattern of *Ubx* gene expression in both embryos and imaginal anlagen. The use of anti-UBX antibodies in leaky alleles of the *Ubx* gene reveals a correspondence between the UBX pattern in the imaginal anlagen and that of the histotypic homeotic transformation in the adult cuticule (WHITE and WILCOX 1985b; WHITE and AKAM 1985; CABRERA, BOTAS and GARCÍA-BELLIDO 1985; BOTAS, CABRERA and GARCÍA-BELLIDO 1988). Therefore, the different patterns of expressivity and spatial specificity of the mutant phenotype in adult cuticule may be correlated with patterns of gene expression in proliferating imaginal cells, opening the possibility of studying the genetic and developmental conditions that determine spatial specificity of *Ubx* gene expression during development.

Contrabithorax (*Cbx*) alleles of the *Ubx* gene cause expression of the gene outside its normal realm of action. They show homeotic transformations of the mesothorax (T2) toward the metathorax (T3) (LEWIS 1955, 1978, 1982), associated with the ectopic presence of UBX in mesothoracic imaginal discs (WHITE and WILCOX 1985b; WHITE and AKAM 1985; CABRERA, BOTAS and GARCÍA-BELLIDO 1985; CASTELLI-GAIR, MICOL and GARCÍA-BELLIDO 1990). These *Cbx* alleles differ in penetrance, in expressivity and, more interestingly, in spatial specificity. Variations in spatial specificity are correlated with variations in the pattern of UBX expression as seen in imaginal discs. In most *Cbx* alleles the nature and location of the associated DNA aberration in the molecular map of the *Ubx* gene is known (see BENDER *et al.* 1983, 1985; Figure 1). However,the spatial specifities of the phenotypes do not follow straightforwardly from the *cis* perturbations in the gene. For one thing, spatial specificity is variable and highly erratic in some *Cbx* alleles. Moreover, in at least one case, *Cbx*^{1}, it has been shown that the phenotype (compact haltere histotype territories in the wing) is not clonal in origin, *i.e.*, the phenotype does not result from perturbations during develop-

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ment maintained by cell heredity (MORATA 1975). Thus, at least in the *Cbx*^{1} case, we must invoke cell to cell interactions to explain the phenotype (CABRERA, BOTAS and GARCÍA-BELLIDO 1985; BOTAS, CABRERA and GARCÍA-BELLIDO 1988). Since the UBX are thought not to diffuse between cells, the dependence on cell interactions demands a consideration of the effect of *trans*-regulatory signals defining spatial specificity, in addition to the affected *cis* controls of the gene.

We analyze in this paper the genetic and developmental conditions that modulate the spatial specificity of phenotypes of *Cbx* alleles. This work is facilitated by our knowledge of the proliferation dynamics of both wing (GARCÍA-BELLIDO and MERRIAM 1971) and haltere imaginal discs (MORATA and GARCÍA-BELLIDO 1976), corresponding to the "autotypic" (wing) and "telotypic" (haltere) (GARCÍA-BELLIDO 1977) conditions of the wing toward haltere homeotic transformation. This approach differs from that of studying haltere toward wing homeotic transformations in loss-of-function alleles of the *Ubx* gene. Such studies have shown that *Ubx* gene activity is cell autonomous in genetic mosaics, *i.e.*, in mosaics resulting from the generation of homozygous *Ubx* mutant cells in a heterozygous, phenotypically normal haltere anlage. In these mosaics there is a confrontation between different histotypes (normal haltere cells and mutant cells transformed toward wing) as a result of their different genotypes. In the case of the *Cbx* alleles, mosaicism is due to changes in the state of activity of the *Ubx* gene during proliferation in cells of the same genetic constitution ("apogenetic mosaics," see GARCÍA-BELLIDO 1986). Therefore we are in this study closer to understanding the signals that may modify *Ubx* gene expression in its postulated role of changing the mesothoracic (wing) ground state into the metathoracic (haltere) one (LEWIS 1963).

Our results suggest that the spatial specificities of the phenotypes of different *Cbx* mutations are due to changes in the proliferation dynamics of normal wing cells and of those transformed toward haltere, changes associated with alterations in timing of *Ubx* gene expression and with interactions between cells of both alternative developmental pathways. This study reveals in addition the existence of novel morphogenetic processes involved in wing pattern formation.

MATERIALS AND METHODS

Mutations: The genetic variants used in this work have been previously described in LINSDLEY and GRELL 1968 and in other references in the text.

Cell density measures: We have estimated the number of cells of each histotype in transformed wings by measuring trichome densities per surface unit. In the normal haltere the number of trichomes per surface unit was considered standard (value of 1, corresponding to 62 cells). In the wing (or wing trichome regions), which has lower trichome densities, the surface unit used was five times larger (including 25 to 15 trichomes, depending upon the region considered). Density values in mosaic spots are given as a fraction of the standard density of the haltere. More than 20 transformed wings (or halteres) of each genotype in Table 1 were drawn in camera lucida. Since trichome density is not constant, the estimation of the number of cells was corrected for densities. In those transformed spots where density transitions of wing to haltere were gradual, the border under consideration has an uncertainty of less than 6–10 cells.

Immunofluorescence staining: We have monitored the spatial distribution of UBX in wing imaginal discs using the FP3.38 monoclonal antibody as described in WHITE and WILCOX 1985a.

Clonal analysis: The cell lineage analysis (GARCÍA-BELLIDO 1975) of the Cbx transformations in wings was done using *mwh jv* as cell markers for trichomes and chaetae, respectively, in mitotic recombination clones induced in *mwh jv Cbx/M(3)i*^{55} or in *mwh jv Cbx/+* heterozygotes. The resulting clones in *mwh jv Cbx/M(3)i*^{55} individuals are *Minute*$^{+}$ with a higher proliferation rate than nonrecombinant cells (MORATA and RIPOLL 1975). Larvae were irradiated with 1000 R (Philips MG 51 Be, 250 rpm, 100 kV, 15 mA, 2 mm Al filter and a FO distance of 20 cm) at different ages (24–48, 48–72, 72–96 and 96–120 hr after egg laying). Since the genotype *Cbx*^{2RM}/+ has weak penetrance, we have used *Cbx*^{2RM}/*Tp(3;1)P115* flies in which penetrance is increased by disrupted transvection (MICOL and GARCÍA-BELLIDO 1988).

RESULTS

Genetic analysis of *Cbx* alleles

Molecular perturbations in the *Ubx* gene associated with the different *Cbx* mutations studied here have been described previously (Figure 1), with the exceptions of *Cbx*^{M1} (BOTAS, CABRERA and GARCÍA-BELLIDO 1988), and the spontaneous mutations that cause the reversion of both *Cbx*^{2} toward *Cbx*^{2RM} (mislabeled *Cbx*^{2} in MICOL and GARCÍA-BELLIDO 1988 and in BOTAS, CABRERA and GARCÍA-BELLIDO 1988) and *Cbx*^{1} toward *Cbx*^{1RM}. *Cbx*^{1} corresponds to a transposition of part of the bxd region to the second intron of the Ubx transcription unit (BENDER *et al.* 1983; O'CONNOR *et al.* 1988). Its spontaneous revertant *Cbx*^{1RM} is not associated with visible chromosomal aberrations (MICOL and GARCÍA-BELLIDO 1988). All the remaining *Cbx* mutations are associated with chromosomal rearrangements with one breakpoint in the *Ubx* gene. These breakpoints map either to regions upstream of the Ubx transcription unit or to the 3′ untranslated region of the last exon of the Ubx unit (*Cbx*^{3} and *Cbx*^{Twt}).

Some features of the adult mutant phenotypes of most *Cbx* mutations have been described previously (LEWIS 1978, 1982; MORATA 1975; CASANOVA, SÁNCHEZ-HERRERO and MORATA 1985; WHITE and AKAM 1985; CABRERA, BOTAS and GARCÍA-BELLIDO 1985; MICOL and GARCÍA-BELLIDO 1988; BOTAS, CABRERA and GARCÍA-BELLIDO 1988). Wing toward haltere transformations, the dominant phenotype associated

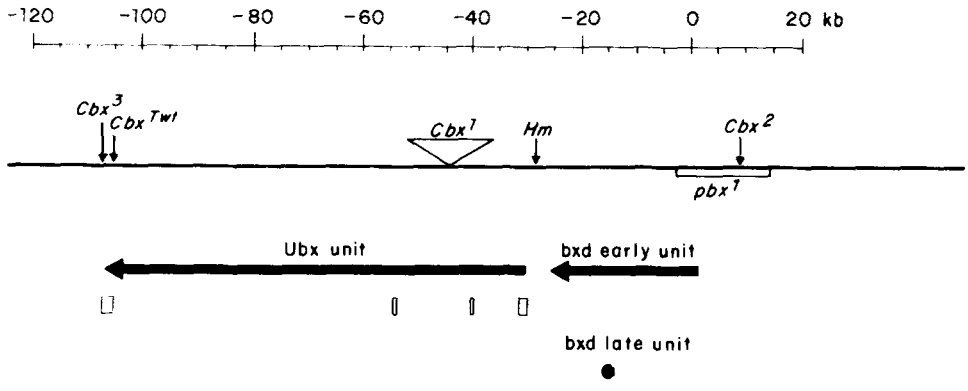

FIGURE 1.—Molecular map of the *Ubx* gene showing mutations used in this work. Transcription units are indicated as heavy arrows. Exons of the Ubx unit of transcription appear as open boxes. Vertical arrows indicate breakpoints. The Cbx^1 insert corresponds, in inverted orientation, to the DNA region indicated as the pbx^1 deletion (open box in the bxd region). The exact breakpoint locations of Cbx^3 and Cbx^{Twi} with respect to the Ubx unit transcripts are not known to the nucleotide level, but they both interrupt the untranslated region of the last exon. Modified from BENDER *et al.* 1983, 1985; O'CONNOR *et al.* 1988.

with *Cbx* mutations, differ in an allele-specific manner in penetrance, expressivity and spatial specificity (Table 1; Figure 3). Their spatial specificities may be either constant or variable in different wings of the same genotype. These variations can follow a spatial order ("variable") or be variegated or "erratic" (GARCÍA-BELLIDO and CAPDEVILA 1978) (Table 1 and next section of results). The penetrance, expressivity and spatial specificity of the dominant phenotype of some *Cbx* mutations can be modulated by the presence in the genome of the *Ubl* allele of the *RpII215* gene affecting the α-subunit of the RNA polymerase II and therefore transcription efficiency (MORTIN and LEFEVRE 1981; MORTIN, KIM and HUANG 1988), or by the presence of *Minute* mutations, which possibly affect protein synthesis (KAY and JACOBS-LORENA 1987). The effect in both types of combinations is to reduce the Cbx phenotype in the mesothorax. The reduction of Cbx dominant phenotype in $Minute(3)i^{55}$ combinations can be graded as follows: $Cbx^{2RM} > Cbx^2 \approx Cbx^{M1} > Cbx^1 \approx Cbx^{1RM}$ (Cbx^3, Cbx^{Twi} and *Hm* are unaffected). For *Ubl* combinations this reduction follows the order: $Hm > Cbx^{2RM} \approx Cbx^2 \approx Cbx^{M1} > Cbx^{1RM} \approx Cbx^1 \approx Cbx^3 \approx Cbx^{Twi}$.

Cbx dominant phenotypes are additive in spatial specificity in heterozygotes between two different *Cbx* alleles (L. GARCÍA-ALONSO and A. GARCÍA-BELLIDO, unpublished results and this work). The Cbx^2/Cbx^{2RM} genotype is lethal even in the presence of an extra wild-type copy of the *Ubx* gene. *Cbx* homozygotes show, as a rule, higher expressivity of the same mutant wing phenotype. This is the case for Cbx^1 and *Hm* (LEWIS 1982). Cbx^{M1} homozygotes show a wing phenotype similar to that of *Hm*/+ heterozygotes (J. E. CASTELLI-GAIR, unpublished results). We have failed to obtain homozygotes of Cbx^{Twi}, Cbx^2 and Cbx^{2RM}

TABLE 1

Dominant (gain-of-function) and recessive (loss-of-function) mutant phenotypes of *Contrabithorax* alleles

	Cbx^1	Cbx^{1RM}	Cbx^2	Cbx^{2RM}	Cbx^3	Cbx^{Twi}	Cbx^{M1}	*Hm*
Breakpoints outside BX-C[a]			91C-E	91C-E	89A	87E-F	84F-85A	—[b]
Dominant phenotype (wing)[c]								
Penetrance[d]	100	99	100	6[e]	80	20	100	100
Expressivity in compartment[f]	C	C	C	V	V	V	C	C
Spatial specificity[f]:								
Anterior	V	V	Ve	WT	Ve	Ve	C	C
Posterior	C	C	C	V	WT	WT	C	C
Recessive, loss-of-function phenotype[g]: compartments affected	T3, (A1a)	T3, A1a	T3, A1a	T3, A1a	(T3a)	WT	T3p, A1a	(T3p), A1a

[a] For those *Cbx* alleles associated to rearrangements one breakpoint maps in the bithorax complex (BX-C), the second breakpoint is given in the table.

[b] Associated to a complex rearrangement with new order: 21-29|88F-61; 100-89E3|32-29|89E2-88F|32-60 (LEWIS 1982).

[c] Phenotype in *Cbx*/+ individuals. Dominant phenotype in notum and mesothoracic leg is not considered in this work.

[d] Penetrance is given as percentage of wings with mutant phenotype.

[e] Penetrance dependent on transvection (MICOL and GARCÍA-BELLIDO 1988).

[f] Expressivity and spatial specificity can be constant (C) in all individuals or variable (V); spatially erratic specificity is designated as Ve; WT indicates wild type phenotype.

[g] T3 indicates transformation of T3a (anterior) and T3p (posterior) towards T2a and T2p. A1a indicates transformation of A1a toward T3a. Parentheses indicate weak transformations. All the loss-of-function phenotypes studied in Cbx/Ubx^{195} heterozygous being compared with $+/Ubx^{195}$ individuals. Since Cbx^{1RM}/Ubx^{195} genotype is lethal, the phenotype was studied in $Cbx^{1RM}/abx^1bx^3pbx^1$ flies.

alleles, even in the presence of an extra dose of Ubx^+ (which may be due to the lethality of the second breakpoint of the rearrangement).

Allele specificity can also be ascertained in the associated recessive, loss-of-function phenotype of *Cbx* mutations, which can be studied in heterozygotes between *Cbx* alleles and loss-of-function mutations in the *Ubx* gene. Study of these recessive effects may help to ascertain the nature of the misregulation that causes the Cbx mesothoracic phenotype. With the exception of Cbx^{Twt}, all the *Cbx* alleles studied in this work have a recessive loss-of-function phenotype, as can be shown in *Cbx* heterozygotes with null alleles of the *Ubx* gene (Table 1), confirming published data for Cbx^1 (LEWIS 1955, 1982; CASANOVA, SÁNCHEZ-HERRERO and MORATA 1985), Cbx^2 and *Hm* (LEWIS 1982), Cbx^{M1} (BOTAS, CABRERA and GARCÍA-BELLIDO 1988), Cbx^{1RM} and Cbx^{2RM} (MICOL and GARCÍA-BELLIDO 1988). Cbx^3 has a weak recessive phenotype, affecting weakly the anterior haltere. Cbx^{M1} and *Hm* have recessive components similar to those observed in *bxd* mutations, with absence of first abdominal tergite and transformation of posterior metathorax toward posterior mesothorax. In Cbx^{M1} this effect is strong in haltere and intermediate in the metanotum; in *Hm* it is very weak in the haltere and intermediate in the metanotum. Cbx^1, Cbx^2 or Cbx^{2RM} heterozygotes exhibit transformations affecting both anterior and posterior haltere (intermediately transformed to wing in Cbx^1 but weakly in Cbx^2 and Cbx^{2RM}).They show, in addition, a reduction of the first abdominal tergite which is almost complete in Cbx^2 and Cbx^{2RM} but only weak in Cbx^1. The Cbx recessive, loss-of-function phenotypes differ not only in the A (anterior) and P (posterior) compartments affected, but also in their proximal (Pr) *vs.* distal (Ds) effects. Some *Cbx* alleles affect haltere but not metanotum (Cbx^1, Cbx^2, Cbx^3 and Cbx^{2RM}), while *Hm* affects metanotum but only weakly the haltere. Thus, clearly the regions and patterns affected by the *Cbx* alleles in the mesothorax do not correspond to those affected in the metathorax, neither do they represent their spatial complements.We will examine in what follows the homeotic transformation in the wing part of the anlage.

Pattern parameters of the Cbx wing mutant phenotypes

Comparison of wing and haltere: Figure 2 shows the pattern features of wing and haltere considered in this work. It includes the topographical distribution of pattern elements (chaetae, sensillae and veins) as described in FERRIS 1950. The borders of homologous compartments in haltere and wing [A/P (anterior/posterior) and D/V (dorsal/ventral) boundary] were ascertained by clonal analysis (GARCÍA-BELLIDO 1975) using the Minute technique. The location of clonal patches of wing patterns in halteres resulting from mitotic recombination in bx^3 (and Ubx^1) heterozygotes (MORATA and GARCÍA-BELLIDO 1976) allows one to infer pattern homologies between wing and haltere. In the Cbx wings we can analyze the opposite condition, namely haltere patches in a context of wing territories [see MORATA (1975) for Cbx^1].

Table 2 gives the number of cells calculated from the densities of trichomes (each corresponding to a single epidermal cell) in the different compartments of both wing and haltere structures (see MATERIALS AND METHODS and MORATA and GARCÍA-BELLIDO 1976). The same table includes the number of cells in anterior and posterior halteres of bx^3 and pbx^1 hemizygotes for comparison. We see in both genotypes that in the transformed haltere the number of wing cells is lower and that of haltere cells higher than that expected from a complete compartment transformation. This finding indicates that the presence of both wing and haltere territories in the same anlage (imaginal disc) may affect the dynamics of their proliferation. We will encounter this phenomenon in the following analysis of the Cbx phenotypes in the wings.

The sizes and cell densities of the transformed territories: As seen above different *Cbx* alleles vary in their allele-dependent parameters of penetrance, expressivity and spatial specificity. These differences could result from different levels of *Ubx* gene activity in different regions of the anlage. These allele-specific variations could in turn be related to the molecular nature of each *Cbx* mutation, such as a promotor perturbation, a new regulatory DNA sequence adjacent to the *Ubx* gene in a rearrangement, etc. These explanations would transfer the problem of pattern specificity to that of the particular perturbation in the *Ubx* gene. However, the present results suggest that the three parameters (penetrance, expressivity and specificity) are modulated by developmental properties of the growing anlage, cell-cell interactions, clonal behavior and temporal effects.

The wings in different *Cbx* mutations have distinct haltere territories (Figure 3) that can be distinguished from the wing territories by trichome size, cell density and background pigmentation. These differences are only clear between the wing spread and the capitellum but not in the wing base. Based on these features we can unambiguously distinguish haltere territories appearing in the wing blade (the distal compartment); they correspond to trichome densities higher than 0.20 (being on average 0.05 in the normal wing and 1.0 in the normal haltere). As we will see below, the transition from wing to haltere territories is abrupt in some *Cbx* alleles but gradual in others. This gradation, however, does not preclude the proper assignment of territories, except along the histotypic borders where a region only a few cells in width may be ambiguous (but see below).

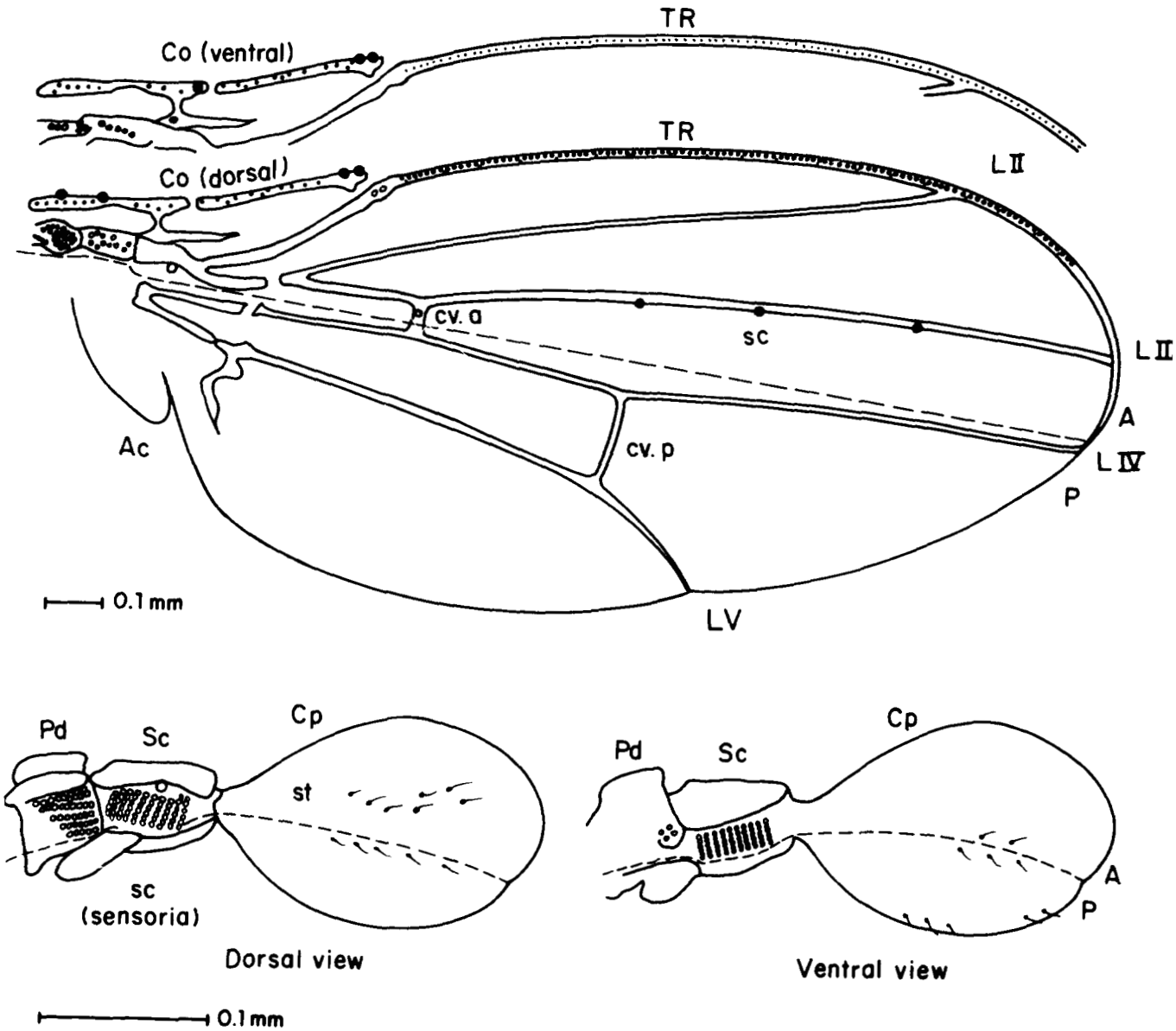

FIGURE 2.—Vein and chaeta pattern of the adult wing and haltere of wild-type flies in their dorsal and ventral aspects. Dashed lines separate territories of the A (anterior) and P (posterior) compartments. A/P boundary in the wing after GARCÍA-BELLIDO, RIPOLL and MORATA (1973), and in the haltere after GARCÍA-BELLIDO (1975) and M. A. GONZÁLEZ-GAITÁN and A. GARCÍA-BELLIDO (in preparation). Co, costal; TR, triple row of chaetae; M, medial; D, dorsal; V, ventral; Al, allula; LII-V, longitudinal veins; cv.a and cv.p, crossveins anterior and posterior; Pd, pedicellum; Sc, scabellum; Cp, capitellum; sc, sensilla campaniformia; st, sensilla trichodea.

It holds for all the *Cbx* mutations that the number of haltere cells present in mosaic territories exceeds (1) that expected from the fraction of wing pattern absent and (2) that expected from the fraction of transformed haltere. We see in Table 2 some extreme cases which support this conclusion. This applies to single compartment transformations (A in Cbx^{3} and Cbx^{Twt}, and P in some Cbx^{1} and Cbx^{2RM} flies) associated with total or partial transformations. In these cases the number of haltere cells can be more than 4,000, the total number of the wild type capitellum. It applies also to transformations affecting both A and P compartments (Cbx^{1}, Cbx^{2}, Cbx^{M1} and *Hm*) where the sum total of haltere cells is larger than 4,000. On the other hand, the same Cbx wing phenotypes show a reduction relative to the expected number of nontransformed wing cells. This reduction in the number of wing cells could be due either to failures in the proliferation dynamics of cells of this histotype or to extrusion of the wing cells from the haltere territory due to negative cell affinities between haltere and wing cells (GARCÍA-BELLIDO and LEWIS 1976; MORATA and GARCÍA-BELLIDO 1976). However, this reduction also occurs in transformations that affect only one compartment, with the other wing compartment having less than the expected 10,000 cells. These results clearly indicate that wing and haltere patterns do not result merely from transformation of wing cells into haltere cells in the mature discs at the end of the proliferation period. They suggest that the final phe-

TABLE 2

Presence of wing and haltere histotypes in wild-type and mutant wing and haltere anlagen

Cases	Number of cells with a given histotype (Wing/Haltere)			Cell density	
	Anterior compartment	Posterior compartment	Anterior and posterior compartments	Wing histotype	Haltere histotype
Haltere					
+/+	0/3000	0/1000	0/4000		1.0
$bx^3/Df(3R)P9$	5000/0	0/2500	5000/2500	0.05	1.0
$pbx^1/Df(3R)P9$	0/6500	5000/0	5000/6500	0.05	1.0
Wing					
+/+	10000/0	10000/0	20000/0	0.05	
$Cbx^1/+$	6400/0	0/3500	6400/3500	0.05	0.5–0.6
$Cbx^2/+$			1200/11000	0.10	0.5–1.0
$Cbx^2/+$			0/8500		0.5–1.0
$Cbx^{2RM}/+$	10000/0	2270/1500	12270/1500	0.05	0.6
$Cbx^3/+$	10000/300	10000/0	20000/300	0.05	0.2
$Cbx^3/+$	500/4500	3000/0	3500/4500	0.05	0.2–0.5
$Cbx^{Twt}/+$	5000/8000	10000/0	15000/8000	0.05	0.2–0.5
$Cbx^{M1}/+$			5000/5800	0.05	0.5–0.9
$Hm/+$			0/5000		0.4–0.7

Figures of number of cells were obtained from individual representative cases (two extreme cases in Cbx^3). These calculations give a different number of wild-type cells than those from direct counting (which is of 30,000 in the wing (GARCIA-BELLIDO and MERRIAM 1971) *vs.* 20,000 in our estimations). Cell densities are given relative the density of the wild-type haltere (1.0; see MATERIALS AND METHODS).

notype results from shifts in proliferation dynamics (and cell affinities) from the wing to the haltere mode in cells at different stages of development depending upon the *Cbx* allele considered.

The analysis of cell densities in the transformed territories is indicative of another feature of *Cbx* mutations, namely that the differentiated trichomes can be intermediate between those of haltere and wing. Compared with the more or less homogeneous cell density of the haltere (1.0) the patches of haltere territories in *Cbx* wings are not homogeneous in trichome density (Table 2). As a rule there is an inverse correlation between trichome density and trichome length. As mentioned above some *Cbx* mutations (Cbx^2, Cbx^3, Cbx^{Twt}) have sharp borders between wing and haltere histotypes within the same compartment. This observation invalidates the interpretation that the smooth transitions seen in the other *Cbx* alleles are due to mechanical expansion by tensions between haltere and wing differentiating cells. Cbx^3 and Cbx^{Twt}, which exhibit abrupt mosaic borders, nevertheless display varied cell densities within haltere territories, with these differences in density changing stepwise along concentric lines. In these mutants the maximal density varies from 0.80 (Cbx^3) and 0.97 (Cbx^{Twt}) in inner regions to 0.20 in the borders. In the other *Cbx* alleles with more gradual transitions, densities also range from 0.97 to 0.32. In Cbx^2, *Hm* and Cbx^{M1} there is a decreasing A to P gradient in cell density superimposed upon another from the center to the periphery in the spots. Both differences, in density and trichome length, probably reflect intermediate states in the homeotic transformation caused by variable amounts of *Ubx* gene expression.

Dorsoventral symmetries in the transformation: A remarkable characteristic of all the *Cbx* mutations analyzed is the strong correspondence between the location and extent of the transformed territories at both wing surfaces (Figure 4). This D/V symmetry is especially clear when we consider the borders of the transformations. Transformations within the A compartment have, as a rule, sharper borders than those within the P compartment. Sharp borders are characteristic of *Cbx* alleles with erratic spots (wing spots in Cbx^2 and haltere spots in Cbx^1 in A compartment, or Cbx^3 and Cbx^{Twt}). But sharp borders may also occur in Cbx^{M1} in the A compartment and all transformations which stop at either the A/P or the D/V compartment boundaries (Figure 8d).

Dorsoventral symmetry is frequent when spots include the D/V compartment boundary (always the case in posterior transformations) but can also apply to isolated spots not contacting the border. This D/V symmetry appears not only in *Cbx* alleles with constant spatial specificity but also appears in those alleles with erratic spatial transformation. In those genotypes with erratic patches in the wing (Cbx^2, Cbx^3, Cbx^{Twt} and Cbx^1 in A), the D/V symmetry applies to the borders of whole mosaic patterns, or at least to large stretches of it. Asymmetries are, on the other hand, found in these alleles mainly in the anterior wing margin, associated more frequently with transformations in the dorsal than in the ventral wing surface.

These cases of asymmetric transformations strongly indicate that the symmetry found does not mechanically result from apposition of both wing surfaces after wing evagination at metamorphosis. In fact, the symmetrical pattern of transformation in dorsal and

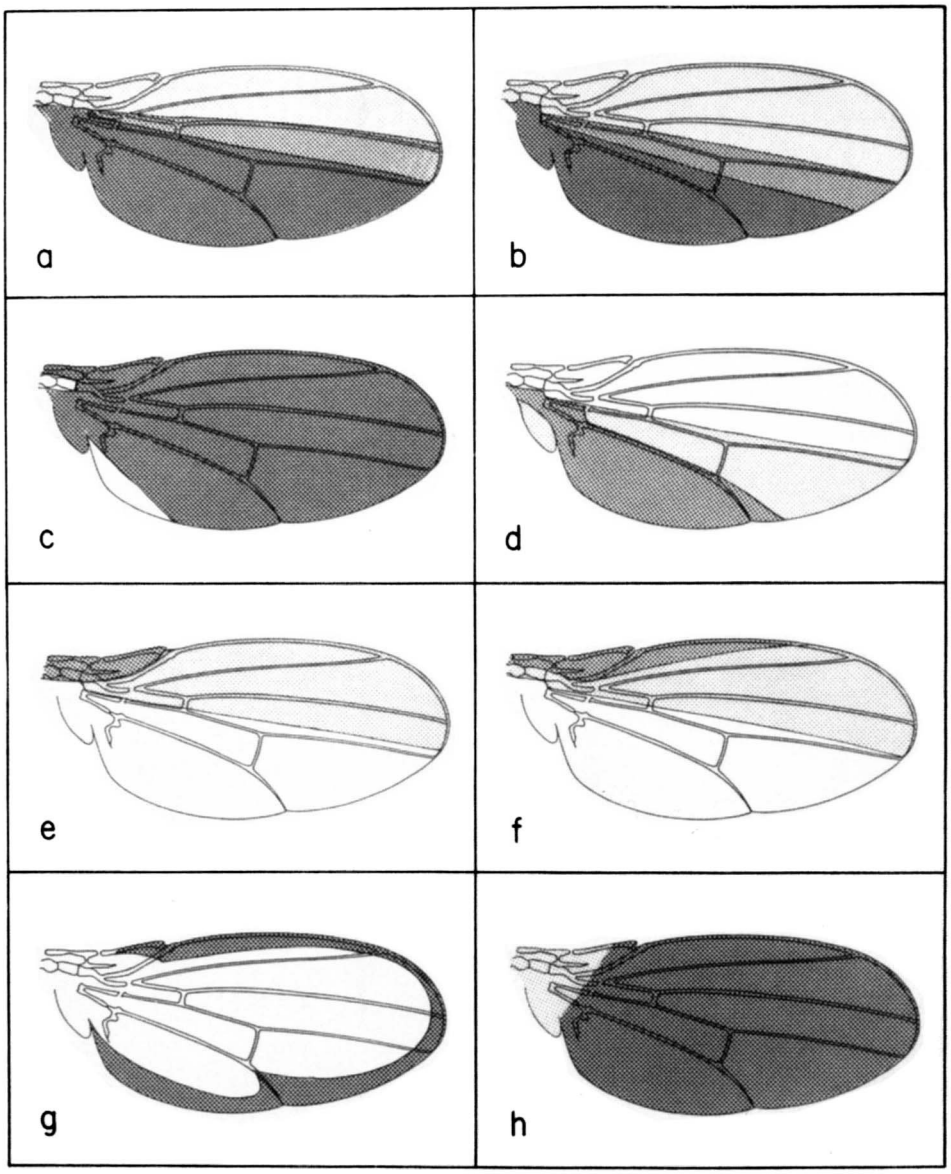

FIGURE 3.—Representation of wing regions transformed by the different *Cbx* alleles in heterozygous condition. Different densities of dots represent, in four classes (0%; 0–50%; 50–90%; 90–100%) the frequency of transformation in any given point of the wing pattern of the population of transformed wings. These distributions are dorsoventral symmetric. (a) $Cbx^{1}/+$; (b) $Cbx^{1RM}/+$; (c) $Cbx^{2}/+$; (d) $Cbx^{2RM}/Tp(3;1)P115$; (e) $Cbx^{3}/+$; (f) $Cbx^{Twt}/+$; (g) $Cbx^{M1}/+$ and (h) $Hm/+$.

ventral surfaces found in trichome territories in the adult wing is correlated with the pattern of expression of UBX, visualized by immunofluorescence staining, in imaginal discs (Figure 5). These UBX patterns have been described for Cbx^{1}, Cbx^{2RM}, Cbx^{3}, Cbx^{M1} and *Hm* (WHITE and AKAM 1985; CABRERA, BOTAS and GARCÍA-BELLIDO 1985; BOTAS, CABRERA and GARCÍA-BELLIDO 1988). We include in our analysis wing discs of Cbx^{1RM}, Cbx^{2} and Cbx^{Twt} heterozygotes. As shown in Figure 5 the stained territories have mirror-image spots symmetric along the D/V compartment boundary in both the A and P compartments. They show, in addition, allele specific regions of staining in the proximal part of the wing and notum. We may again notice that the boundaries between stained and non-stained territories are sharp, as reported before for other mutations in the *Ubx* gene (CABRERA, BOTAS and GARCÍA-BELLIDO 1985; BOTAS, CABRERA and GARCÍA-BELLIDO 1988). For obvious reasons, in *Cbx* alleles of erratic spatial specificity it is not possible to correlate the pattern of the adult transformation and that of the UBX in discs, but the shape and topology of the spots corresponds well between adult and the fate map of the discs (CABRERA, BOTAS and GARCÍA-BELLIDO 1985). The restriction of the UBX pattern to either the A or P compartment is seen in the discs as a discontinuity along a constant (A/P) boundary (Figure 5, e and f).

Cuticular patterns in transformed regions: The haltere territories appearing in the Cbx wings have been defined so far only by the presence of haltere trichomes. These territories may contain, in addition, sensory elements characteristic of the haltere such as sensilla trichodea (s.t.) and sensilla campaniformia (s.c.). However, we find typical wing pattern elements included in these territories, even in total haltere transformations. We analyze these patterns in that which follows.

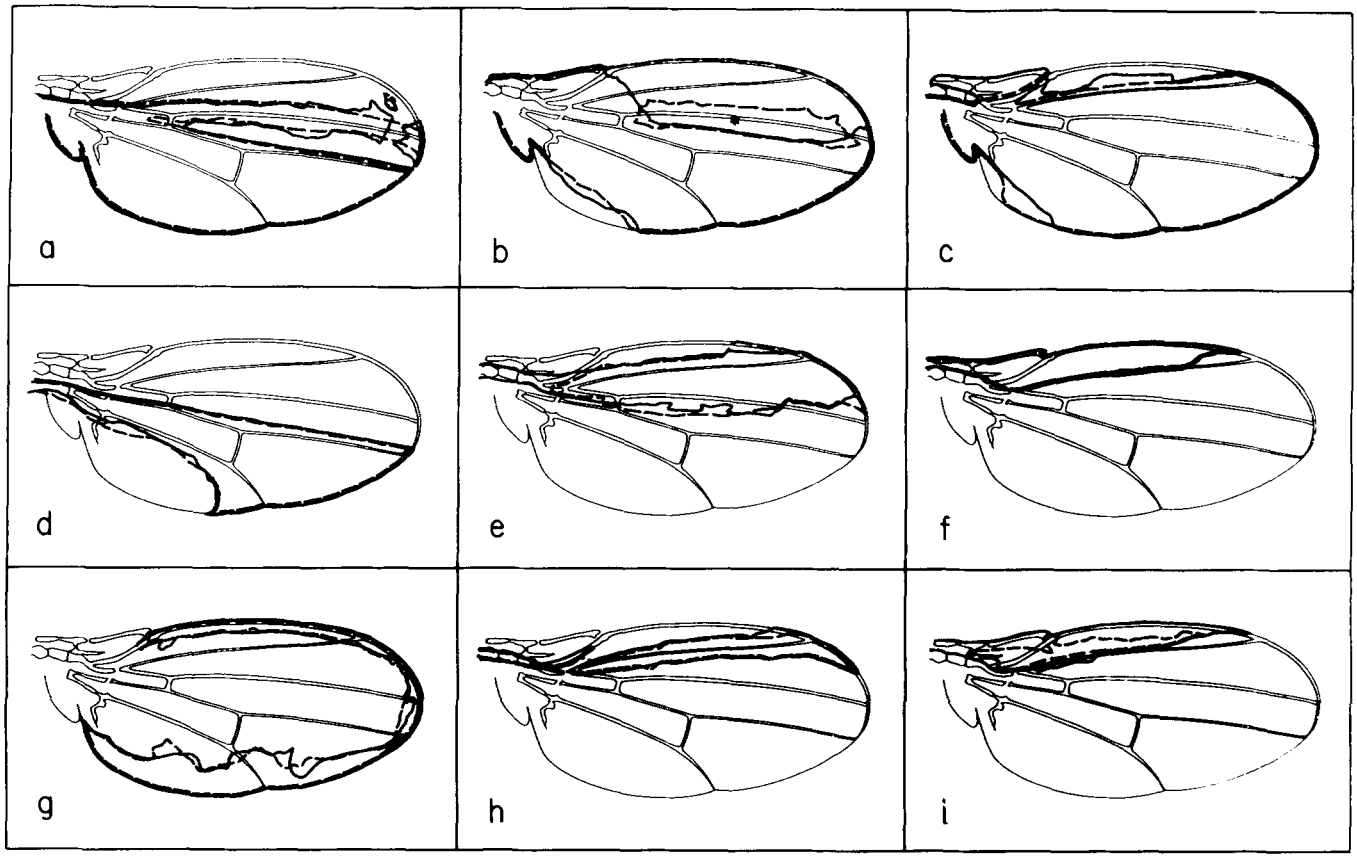

FIGURE 4.—Representative examples of symmetric transformations in the dorsal and ventral wing surface of different *Cbx* heterozygotes. (a) Cbx^1/+; (b and c) Cbx^2/+; (d) $Cbx^{2RM}/Tp(3;1)P115$; (e and f) Cbx^3/+; (g) Cbx^{M1}/+; (h and i) Cbx^{Twt}/+. Continuous lines of patches represent borders in the dorsal, dashed lines in the ventral surface. Outlined territories include haltere trichomes except in b where * corresponds to an island of wing cells surrounded by haltere trichomes.

Haltere sensillae differentiate exclusively within haltere trichome territories, never outside. They usually differentiate to completion in types and pattern arrangements characteristic of different regions of the haltere; s.t. in single rows and clusters and s.c. in regular groups of rows (sensoria) (Figure 2). They may appear also disarranged and in incomplete patterns even in large haltere territories. Moreover, there seems to be no correlation between the number of elements and the size of the patch, with some small haltere territories containing more than the normal number of sensory elements (*e.g.*, in a Cbx^3 spot with 148 cells we have counted 5 s.t.), and some large extensions without sensory elements. The type and pattern of sensory elements depends on the position of the territory in the wing pattern, indicating a general topological homology between both anlagen (Figure 2). There are, however, exceptions to this rule of homeotic substitution. Whereas in the normal capitellum there are 13 dorsal and 6 ventral s.t., in Cbx transformed regions we find on average more sensilla in the ventral surfaces and fewer in the dorsal (*e.g.*, Cbx^1 cases with 13, 11 and 10 ventral *vs.* 1, 4 and 0 dorsal). This applies to A and P transformations in all different *Cbx* alleles, except in *Hm*.

As mentioned above we also find chaetae and sensory elements of the wing immersed within haltere trichome territories (Figures 7 and 8). This holds for all *Cbx* mutations, including *Hm* wings. The remaining wing elements include the triple row (dorsal, medial and ventral chaetae), s.c. of vein LIII, stretches of veins (recognizable by higher trichome density and stronger pigmentation than the surrounding haltere territory), and, more rarely, the posterior double row. The triple row patterns may appear normal, or nearly normal, along short or long stretches (Figures 7 and 8). In large spots of haltere-type tissue, however, the inner parts have fewer of those wing elements. Wing structures may differentiate abnormally, the dorsal chaetae being most commonly affected. Frequently, we find the dorsal and medial rows of chaetae (corresponding to the dorsal wing compartment) separated from the ventral row of chaetae (of the ventral compartment) by stripes of 10–15 haltere trichomes (Figures 8 and 9). This condition may occur in all *Cbx* mutants in cases of partial transformations. Interestingly in cases of asymmetrical D/V transformation along the anterior wing margin (*e.g.*, in Cbx^{Twt} and Cbx^3) the histotypic trichome border may run between the medial + dorsal and the ventral rows (Figure 9).

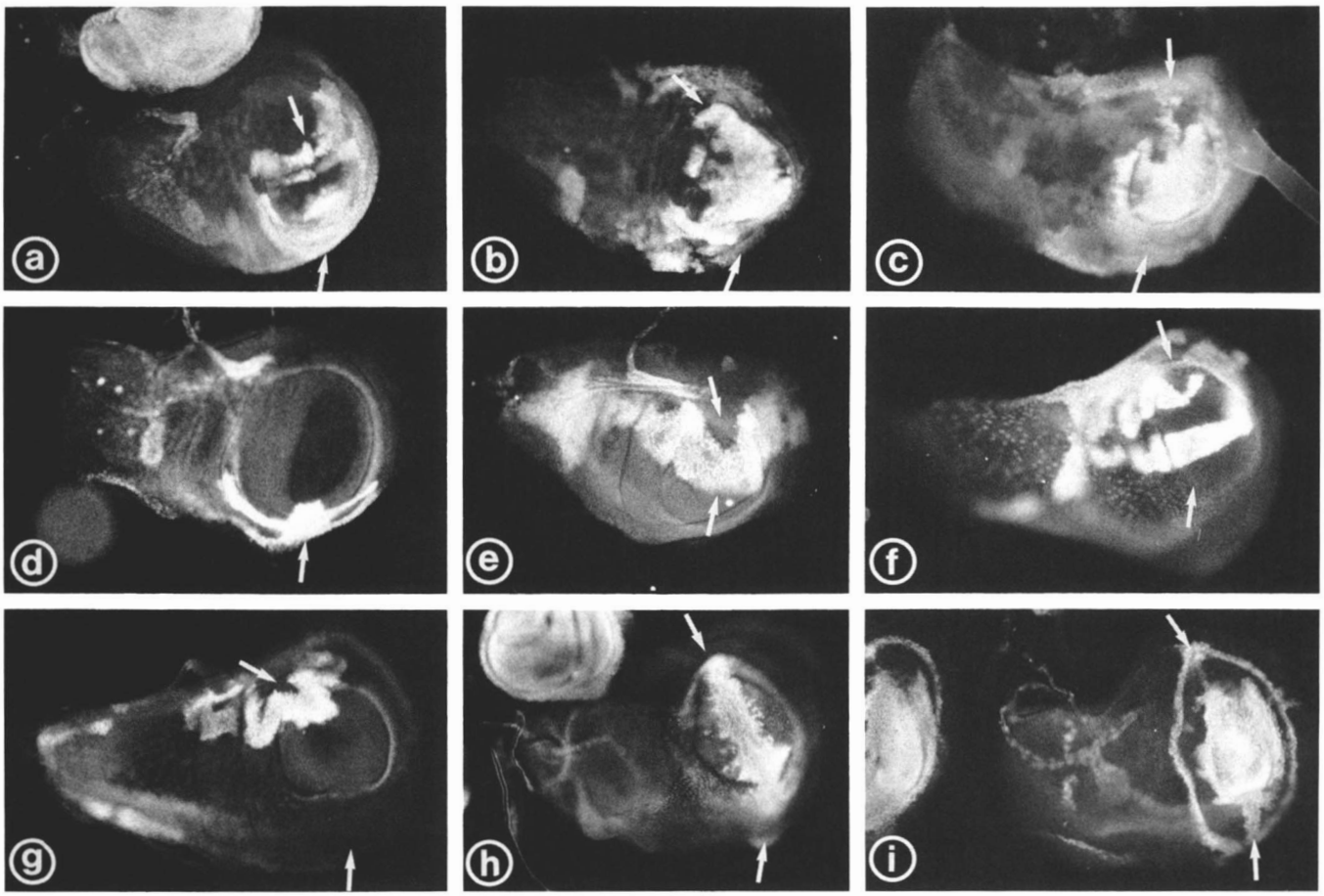

FIGURE 5.—Characteristic patterns of immunofluorescence staining against UBX in wing imaginal discs of: (a) $Cbx^{1RM}/+$; (b and c) $Cbx^{2}/+$; (d) $Cbx^{2RM}/Tp(3;1)P115$; (e and f) $Cbx^{3}/+$; (g) $Cbx^{Twt}/+$; (h) $Cbx^{M1}/+$; (i) $Hm/+$. The arrows point to the dorso ventral compartment boundaries. Observe the symmetric pattern of UBX at both sides of that boundary. All photographs are taken at the same magnification. Sizes of the imaginal discs differ depending on genotype; the reduction with respect to the wild type wing disc (the most similar is $Cbx^{Twt}/+$) denotes the transformation of haltere.

In these cases the elements may be adjacent, as in the normal wing margin. But they may also appear separated: either the dorsal + medial rows in haltere territories away from the histotypic border and the ventral row at the border, or vice versa. Less frequently the border runs outside the triple row elements for short distances. These observations indicate that the differentiation of the triple row elements occurs independently of the actual histotypic differentiation of the trichomes [see MORATA and GARCÍA-BELLIDO (1976) for similar situations in haltere toward wing transformations associated with *bx* alleles].

Cuticular patterns in nontransformed regions: We have seen that the presence in Cbx mutant wings of haltere territories affects the size of the wing territory in other compartments. This effect may be due to (1) interactions between cells of different developmental pathways, or (2) the subthreshold expression of the *Cbx* allele in wing cells, affecting their proliferation dynamics but not their final differentiation as haltere. The second possibility seems unlikely since this phenomenon is found in *Cbx* mutations whose transformations are known to be clonal (*e.g.*, Cbx^{3} and Cbx^{Twt}, see below) or which are (in genetic tests and late patterns of UBX expression) exclusively expressed in one of the two compartments (Cbx^{3}, Cbx^{Twt} in the A and Cbx^{2RM} in the P compartment). It holds, however, for these as well as the rest of the *Cbx* mutants, that the presence of haltere territories may affect the wing pattern in the same compartment or in the opposite one. The effects within the same compartment are various; they may perturb the pattern of triple row elements causing an accumulation of elements proximal to the haltere spot and a decrease of them distally (Figure 8a). They may affect the veins, causing lack of vein stretches distal to the haltere territory. In other cases, extra veins may appear connecting the extant wing veins with the haltere territory, or extra crossveins may appear between normal veins. These effects occur to a lesser degree in regions farther removed from the haltere territories. In cases of D/V asymmetry, when the territory affects only one wing surface, the opposite surface retains the normal veins. These effects may transgress

the A/P boundary in *Cbx* alleles with restricted expression, *e.g.*, lack of vein LIV in Cbx^3 and Cbx^{Twt} wings and reduction of the interval between A/P boundary and LIII in Cbx^{2RM} wings. This happens when the haltere territories are large and close to, or reach, the A/P boundary.

Clonal analysis of Cbx transformations

As seen above *Cbx* mutations differ in topographical specificity, and in some *Cbx* alleles this spatial specificity can be erratic (variegated) between different wings. These allele and region-specific properties beg the question of the clonality of the transformations. Cell lineage analysis of these wings helps to ascertain whether the mosaic patches correspond to clones of cells retaining by cell heredity early or late transformations or, alternatively, whether the patches result from cell interactions affecting the ability of cells to express the *Ubx* gene in particular wing regions. We have carried out these cell lineage studies using the Minute technique (MORATA and RIPOLL 1975). Thus the overgrowth of the $Minute^+$ clones (labeled with *mwh*, a mutation that changes single trichome cell processes into multiple ones in both wing and haltere, and *jv*, a mutation that marks chaetae) can expand committed wing or haltere cells to maximal confrontation along histotypic borders, indicating clonality of the transformation. But even when the transformation is not strictly clonal, overgrown $Minute^+$ clones may expand one or the other histotypic territory, thus breaking the D/V symmetry, and suggesting cell-heritable components in the transformation.

In Figures 6 and 7 we present representative cases of this cell lineage study. Only in the cases of Cbx^3 and Cbx^{Twt} (affecting the A compartment) can we unequivocally state that the haltere transformations are clonal in origin. This statement is based on 9 clones (in 779 Cbx^3 wings showing mutant transformation) and 37 clones (in 947 Cbx^{Twt} wings showing mutant transformation) that appear in either the dorsal or the ventral surface of the wing. In these cases either the *mwh* ($Minute^+$) territory occupies a region of haltere histotype abutting for hundreds of cells with cells of wing histotype, or it appears in wing territories having long borders with the transformed patch. We have found only 3 clones in Cbx^{2RM} (affecting the P compartment) in 554 wings showing mutant transformation, but in all of them the clonal and histotypic restriction holds along hundreds of cells. We have found only 6 clones in 2192 Cbx^2 wings that either included and filled up (4 cases) the rare wing patches immersed in the haltere territory or appeared (2 cases) in the haltere territories contacting the wing patches for tens of cells. These few but unequivocal cases are indicative of clonality. In the Cbx^{M1} wings we have found 27 clones of interest (in 692 wings), of which 10 anterior clones and 7 posterior clones have the *mwh* territory transgressing the histotypic boundary. In 10 cases of posterior patches the clone runs along the histotypic boundary for many cells, although it can contain cells of both histotypes. There is one doubtful case similar to these in the A compartment. In 17 of these 27 cases, the *mwh* ($Minute^+$) territory included in the haltere transformation is associated with large haltere territories breaking the D/V symmetry, suggesting (partial) expansion of the haltere cells in the extra proliferating $Minute^+$ anlage.

In the case of Cbx^1, MORATA (1975) already showed that the transformation in the posterior compartment is not clonal. We have found 30 pertinent clones (in 2197 Cbx^1 wings studied) confirming that conclusion. Interestingly, clones with large territories of haltere histotype may contain a few (6, 14) wing cells. In some cases a clone in the haltere territory limited by wing cells probably corresponding to the anterior compartment (or, reciprocally, anterior clones limited by the haltere histotype) may respect the same A/P boundary. Interestingly posterior transformation limited by the A/P boundary (as judged from the remnant A wing pattern or by borders of $Minute^+$ A clones) are very common in Cbx^1 wings. In other cases posterior clones extend anteriorly beyond the haltere histotype, therefore clearly transgressing the histotypic boundary. Unfortunately we have found only 3 clones exhibiting the rare (especially in a *Minute* background) transformations in the A compartment. All these clones were included in the haltere territory and limited by the wing histotype but only for 20–40 cells, indicating a possible (late) clonality in the A compartment. The topography of 8 clones in 1157 Cbx^{1RM} wings is similar to that found in Cbx^1 indicating likewise in that case a nonclonal transformation. We could not discriminate whether the *Hm* transformation is clonal in the border region where wing and haltere trichomes are indistinguishable (756 wings studied).

Clonal analysis was used, in addition, to investigate the lineage relationships of dorsal + medial and ventral chaetae of the triple row in those haltere territories which retain these wing elements. As shown in Figures 8 and 9 clones can run between both rows when they are adjacent to each other (as in the normal wing margin). In those cases where the rows are separated by stripes of haltere trichomes, the *mwh jv* clones (5 dorsal and 2 ventral) can either include ventral chaeta elements and stop close to the dorsal + medial row or vice versa. We have also found the reciprocal situation in 6 clones (1 dorsal and 5 ventral) where the clone stops immediately in front of either the ventral or the dorsal + medial row. We found one clone passing from one row to the other in the same wing margin but we have not found clones occupying exclusively this stripe of haltere tissue. Moreover this internal stripe separating both rows of

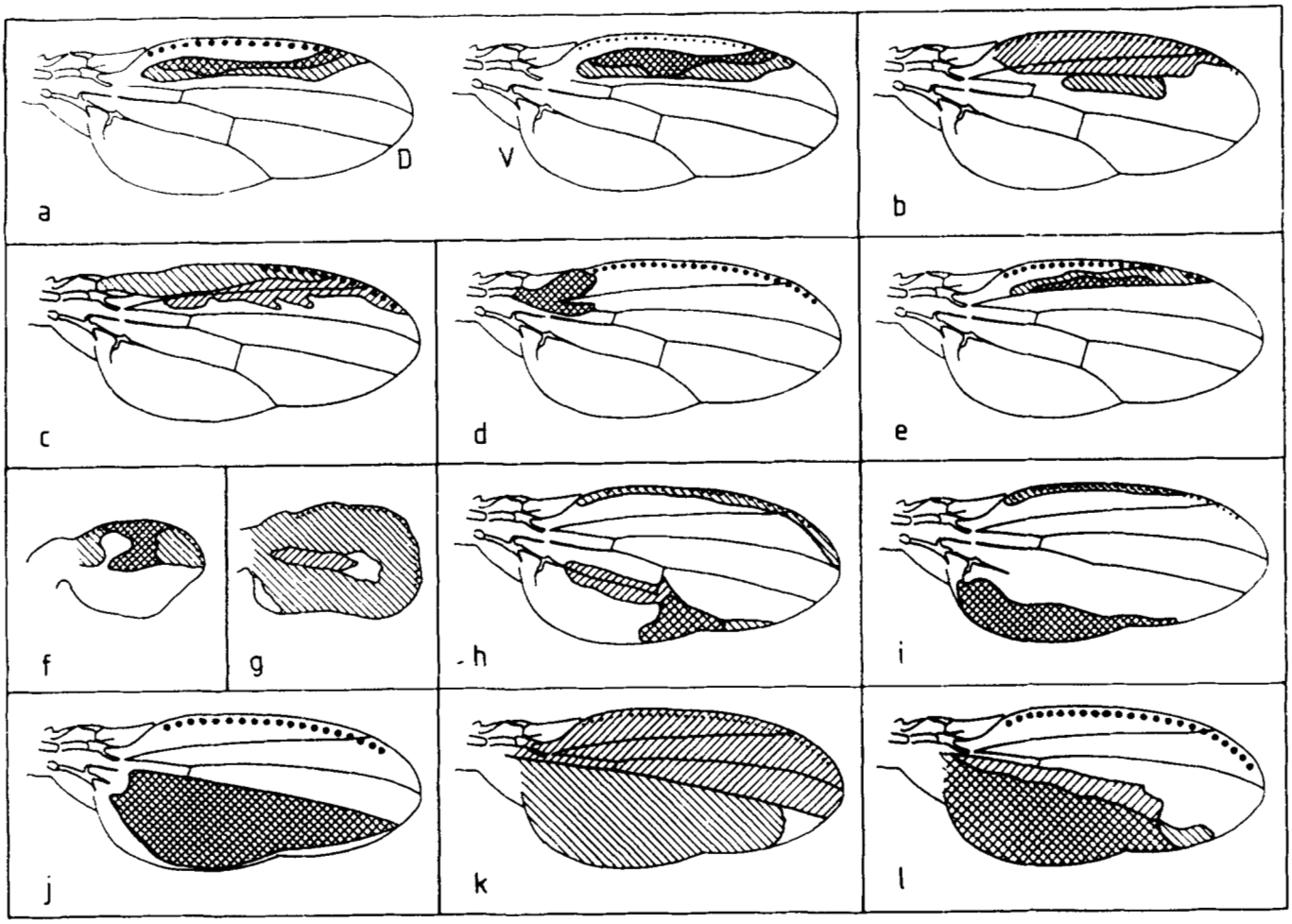

FIGURE 6.—Representative examples of clones of *mwh jv Minute*$^+$ cells in apogenetic mosaics of haltere territories in wing of different *Cbx* alleles. (a) dorsal (D) and ventral (V) aspect of the same Cbx^3/+ wing; (b) Cbx^3/+ wing; (c–e) Cbx^{Twi}/+; (f and g) Cbx^2/+; (h and i) Cbx^{M1}/+; (j) $Cbx^{2RM}/Tp(3;1)P115$; (k and l) Cbx^1/+. The dorsal or ventral aspect can be distinguished by the thickness of the triple row elements (●●●●: dorsal; · · · · ·: ventral). All Cbx wings are morphologically deformed but the clones have been projected upon a normal sized wing using the remaining wing landmarks as reference, except for Cbx^2. ////: *mwh* clone; \\\\: haltere histotype.

chaetae may have large variations in width. Both findings argue against a possible separate lineage for this territory.

DISCUSSION

Proliferation dynamics of the homeotic transformations: By criteria of comparative anatomy the haltere of diptera is a secondary modification of an ancestral metathoracic wing. LEWIS (1963) postulated, based on genetic data, that this modification results from the activity of the *Ubx* gene. Thus *Ubx* loss-of-function mutations transform the haltere into wing, and excess-of-function (derepression) mutations, the *Cbx* alleles, lead to the reciprocal transformation of wing to haltere, suggesting that *Ubx* gene activity generates metathoracic development by suppressing the mesothoracic one. Clonal analyses have shown that the proliferation dynamics of the corresponding imaginal discs (wing and haltere) are very similar. They have the same cell division rate during the two last larval instars but differ in either the number of founder cells in the embryo or the time in which cell proliferation starts in the first instar, so that the final number of cells is about 50,000 in the wing disc (30,000 in the wing proper) and 5,000 in the haltere disc (4,000 in the haltere proper) (GARCÍA-BELLIDO and MERRIAM 1971; MORATA and GARCÍA-BELLIDO 1976; GARCÍA-BELLIDO 1975). As genetic mosaics for *Ubx* mutations show, both the proliferation dynamics and the histotypic differentiation of wing and haltere cells is a cell autonomous property (MORATA and GARCÍA-BELLIDO 1976). By contrast the observable mosaicism in *Cbx* alleles corresponds to variations in the apogenome (GARCÍA-BELLIDO 1985, 1986), *i.e.*, to variations in the state of activity of the *Ubx* gene in cells during development. We therefore designate the Cbx phenotypes as "apogenetic" mosaics. The possibility of monitoring *Ubx* gene expression in cells using antibodies has allowed us to confirm inferences based on the adult phenotype. Whereas UBX appear only in the ventral part of the P compartment (posterior pleura) in wild-type wing discs, various *Cbx* alleles

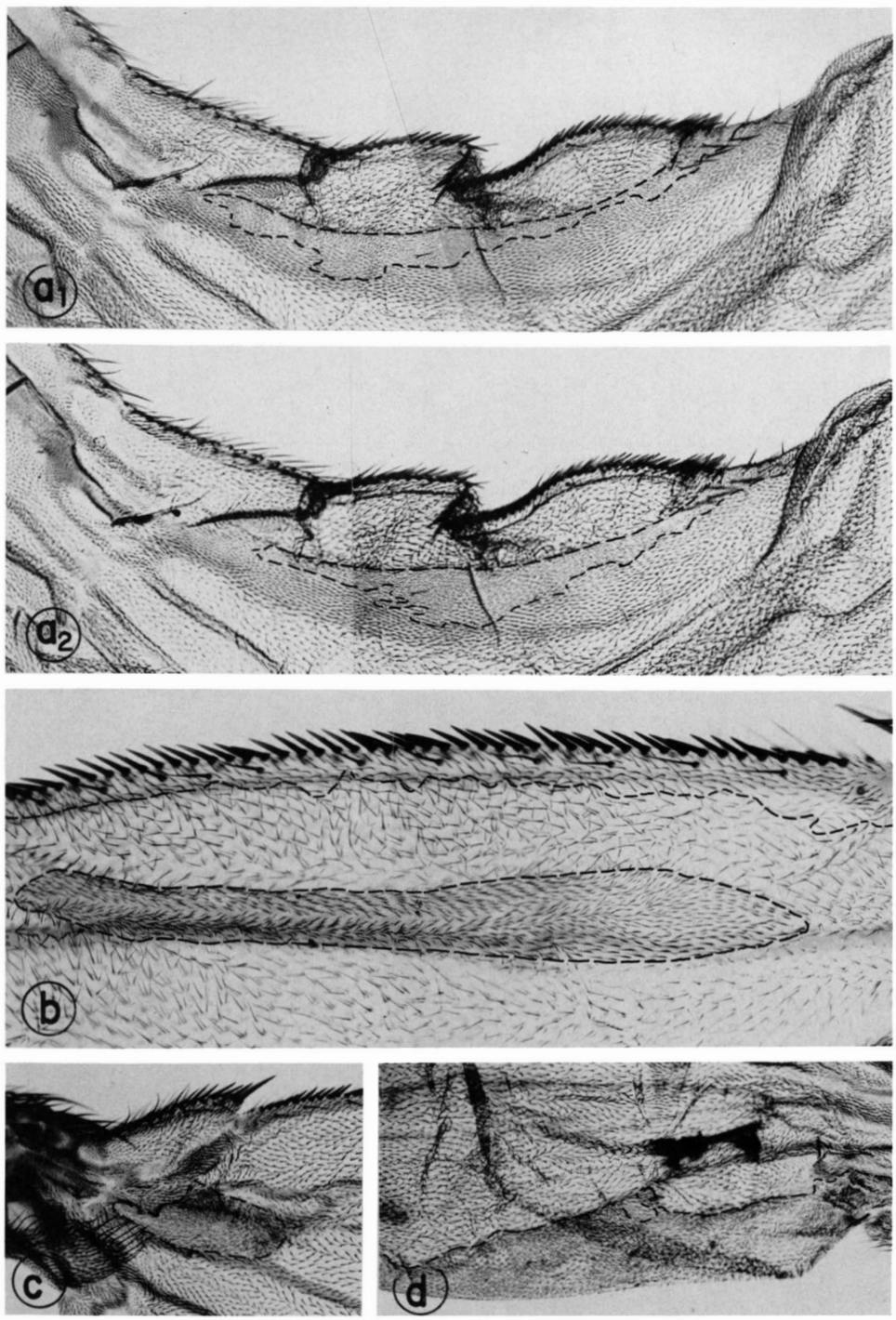

FIGURE 7.—Characteristic examples of *mwh jv* clones in different apogenetic mosaics of (a) Cbx^{3}/+; (b and c) Cbx^{Twt}/+; (d) Cbx^{M1}/+. (a_1 and a_2) dorsal and ventral view of the same wing (observe the dorsoventral symmetry of the transformation). Notice that the borders of the clones coincide with the wing/haltere histotypic borders.

show UBX antibody staining in other parts of the wing discs, in fact in allele-specific regions, corresponding to the cell differentiation pattern of the adult transformation (see references in BOTAS, CABRERA and GARCÍA-BELLIDO 1988).

The present work concerns the developmental conditions that determine these Cbx apogenetic mosaics. We expect that the expression of the *Ubx* gene in the wing cells will affect their proliferation dynamics as well as their final, directly observable, cell differentiation. Therefore the first questions relate to the developmental timing and spatial pattern of the shift from the wing to the haltere mode of cell proliferation. Clonal analysis of the cuticular transformations has revealed that Cbx^{3}, Cbx^{Twt}, Cbx^{2RM} and Cbx^{2} transformations (scoring for Cbx^{2} wing islands in haltere territories) are clonal; *i.e.*, cells in different stages of development acquire (the three former) or lose (Cbx^{2}) the haltere mode and retain the state thereafter by cell heredity. There are indications of late clonality in Cbx^{M1} wings (10/17 clones P, 1/10 clones A) and of expansion (associated with $Minute^{+}$ overgrowth) of transformed territories. By contrast the Cbx^{1} and Cbx^{1RM} transformations in the posterior compartment

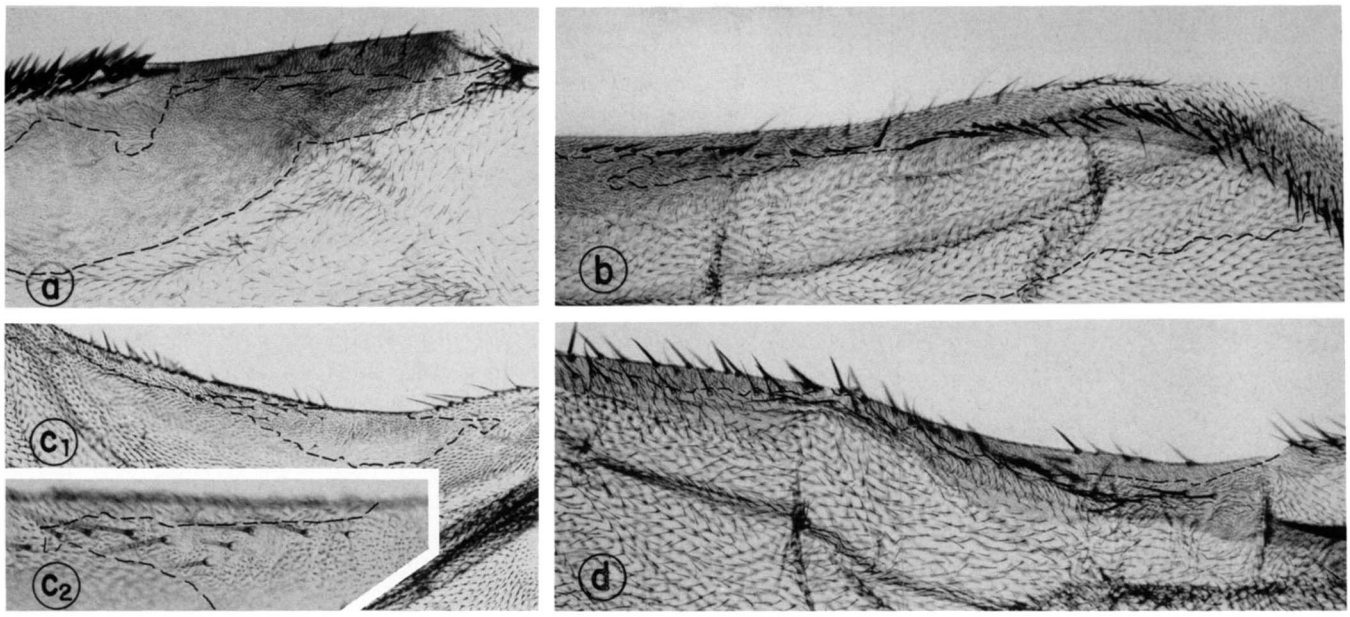

FIGURE 8.—Clonal analysis of the wing anterior margin in Cbx transformations that cause separation of triple row elements (see Figure 9 for schemes). (a) Cbx^{Twt}; (b and d) Cbx^{M1}; (c) Cbx^{3} (c_2 inset of c_1). Notice in (a) concentration of triple row elements proximal (left) to the haltere transformation and histotypic clonality; in (b) an example of lack of histotypic clonality; in (d) histotypic clonality is due to the coincidence with the D/V compartment boundary.

are clearly nonclonal, confirming for the former allele the observations of MORATA (1975). Unfortunately we could not find clones associated with the rare mosaics in the A compartment of Cbx^{1} wings. Neither could we ascertain, due to the lack of distinguishable markers in the base of wing and haltere, the clonality of *Hm* transformations.

In the cases where there is a demonstrable clonality we could, in principle, calculate from the fraction of the wing which is transformed the time of the transformation. However, as discussed below, there are mutual interferences in cell proliferation between haltere and wing cells. Moreover, we cannot estimate from the number of transformed cells the number of its founders and the time of their determination. However, some observations can be made. In clonal *Cbx* alleles the size of the transformation varies widely from nearly the whole wing, possibly resulting from early *Ubx* gene expression in several cells, to just a few cells in late clones. The wings in Cbx^{2} can be almost completely transformed, and can contain very few wing cells (down to 100). Such wing patches are smaller than the expected 1000 corresponding to the minimal, earliest, wing clones (GARCÍA-BELLIDO and MERRIAM 1971). This suggests that they derive from haltere-committed cells that reversed to wing during late stages of cell proliferation. Thus, shifts in cell commitment may possibly occur in these *Cbx* alleles at different times of development (Cbx^{3}, Cbx^{Twt} and Cbx^{2RM}) and be reversible (Cbx^{2}). The erratic phenotypes and shape of patches of all these *Cbx* alleles can thus be explained in terms of this clonality.

In cases of nonclonal transformations, commitment, if any, is clearly reversible. In those Cbx^{1} wings where the wing is almost fully transformed into haltere, with the corresponding decrease in the number of adult cells, the commitment to haltere development must have been very early and must have affected all or most cells. An alternative explanation–massive cell lethality of wing cells due, for example, to cell sorting out (GARCÍA-BELLIDO and LEWIS 1976) at the end of development, in order to reach the normal haltere size–is implausible, because we find wing territories and larger than normal haltere territories in all *Cbx* alleles. Sorting out of haltere and wing disc cells as a cause of the appearance of compact territories could be monitored in clones; the expected split clones under this hypothesis were not observed. Interestingly, in those *Cbx* alleles with non-clonal transformations the transformed territories are topographically adjacent in the disc. This indicates that position prevails upon lineage and suggests that cell communication and recruitment take place between neighboring cells (CABRERA, BOTAS and GARCÍA-BELLIDO 1985; BOTAS, CABRERA and GARCÍA-BELLIDO 1988).

The study of the apogenetic mosaics of the *Cbx* alleles uncovers other features indicative of cell behaviors intermediate between wing and haltere. It is a rule for all *Cbx* alleles (of clonal and nonclonal phenotypes) that transformed territories can contain more cells than expected, whether the transformations are restricted to compartments (Cbx^{3} and Cbx^{Twt} in the A and Cbx^{2RM} in the P compartment) or affect both A and P compartments (Cbx^{2}, Cbx^{1}, *Hm* and

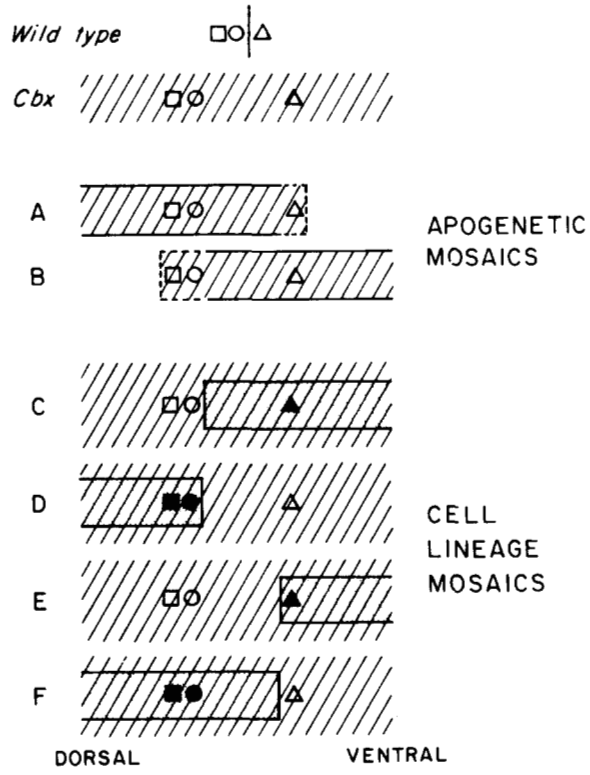

FIGURE 9.—Dorsoventral compartment boundaries in the anterior wing margin of wild type flies and of apogenetic mosaics of haltere territories in wings of *Cbx* mutants. In the latter case the dorsal and ventral chaeta elements of the wing triple row may appear separated by a band of haltere trichomes. The border of the apogenetic mosaics separating wing from haltere trichomes appear as in A (27 cases) or as in B (2 cases). The cell lineage analysis for similar mosaics allows us to ascertain the D/V boundary: the extent and border of the clone is labeled by dots and filled symbols (marked by *mwh jv*). We observed 2 C cases, 1 D, 5 E and 5 F. //// : haltere histotype. In A and B dashed lines indicate uncertainty in the inclusion of chaetae in haltere territory.

Cbx^{M1}) (Table 2). Even in partial transformations they may contain more than the expected 3,000 (A) + 1,000 (P) or 4,000 (A+P) cells of the wild type haltere. This could be indicative of transformations occurring at the end of the cell proliferation period, or to variable degrees of *Ubx* gene derepression. The first alternative would not explain the majority of patches of clonal origin. The second one is supported by another observation that also applies to all *Cbx* alleles studied, namely that the territories defined by haltere trichomes may have lower cell densities and larger trichomes than in the haltere, and may contain, in addition to haltere sensilla, chaetae elements that correspond to wing patterns. These wing elements may appear in territories that are, in terms of trichome phenotype, completely transformed. Comparable mixed mosaics were found also in clones of Ubx^- cells in haltere backgrounds (MORATA and GARCÍA-BELLIDO 1976) where the trichome cells of the clone remained haltere, while some cells appeared transformed into wing chaetae. We interpreted those findings as resulting from differential perdurance of Ubx products: above threshold in cells differentiating as haltere trichomes, and below it in cells that had to respond to new signals in the D/V boundary and differentiate as wing chaetae. The same this phenomenon (perdurance) probably occurs in the Cbx apogenetic mosaics. Thus, intermediate phenotypes are indicative of intermediate levels of *Ubx* gene expression in these apogenetic mosaics. At the population level, intermediate levels may also represent mixtures of individual cells at with full or no *Ubx* gene expression, as suggested by the sharp borders of UBX pattern found in imaginal discs in clonal and nonclonal *Cbx* alleles (Figure 5) as well as in other previously studied mutations in the *Ubx* gene (CABRERA, BOTAS and GARCÍA-BELLIDO 1985; BOTAS, CABRERA and GARCÍA-BELLIDO 1988). The fact that transformed (and nontransformed) territories in nonclonal *Cbx* (and other) alleles appear in compact contiguous patches indicates that cell-cell interactions lead to "recruitment" of next neighbors among reversibly committed cells.

Cell-cell interactions: We have seen in *Cbx* wings that the wing territories may also be abnormal in size and pattern (*e.g.*, in venation and chaetae patterns of the wing margin). These malformations could be due either to the presence of undetectable (in immunofluorescent staining) amounts of UBX in the wing proliferating cells or to perturbations of normal wing development by the presence of neighboring haltere territories. The observation that this situation holds for both clonal and nonclonal transformations argues for the latter explanation. These interactions cannot be due to the diffusion of Ubx products between cells, since *Ubx* genetic mosaics are fully cell autonomous, but can be explained by the phenomenon described as "accommodation." We applied this term to explain mutual adjustment in vein patterns in genetic mosaic borders between both wild-type and mutant cells for vein mutations (DIAZ-BENJUMEA, GONZÁLEZ-GAITÁN and GARCÍA-BELLIDO 1989). It applies also for *engrailed* genetic mosaics (LAWRENCE and MORATA 1976) and it may also apply to apogenetic Cbx mosaics. The proposed short range interactions between mutant and wild-type cells in genetic mosaics or in apogenetic mosaics also explain the remarkable symmetries of transformations found in the present work. We have found frequent symmetries in the extent and borders of haltere transformed territories with respect to the dorsal-ventral compartment boundary, both in anterior and posterior wing margins. This symmetry was found in nonclonal as well as in clonal transformations and in constant as well as in erratic phenotypes (although this symmetry is less clear in Cbx^{Twt} wings). This mirror-image symmetry can also be observed in wing discs in their UBX staining pattern;

therefore, it precedes the apposition of both wing surfaces along the wing margin at metamorphosis. We interpret these symmetries also in terms of accommodation between "wave" functions for cell proliferation that use compartment boundaries as axis of reference, as it seems to apply in the determination of veins symmetrically in both wing surfaces (GARCÍA-BELLIDO 1977; DIAZ-BENJUMEA, GONZÁLEZ-GAITÁN and GARCÍA-BELLIDO 1989).

The detailed analysis of the anterior wing (haltere) margin in the Cbx apogenetic mosaics is relevant to the study of the basis of this symmetry and its consequences in the formation of the triple row pattern of chaetae in the anterior wing margin. We have frequently observed these chaetae elements included in (or bordering) haltere territories in this margin. They may appear in three parallel rows, as in the normal wing, but with the dorsal and medial row being separated from the ventral by a stripe of haltere trichomes; the three rows are immediately adjacent in the normal wing. In the normal wing dorsal+medial chaetae are of dorsal lineage and ventral ones of a ventral lineage, the D/V compartment boundary running between them (GARCÍA-BELLIDO, RIPOLL and MORATA 1973). In *Cbx* apogenetic mosaics the rows appear separated when both or one of the rows are immersed in haltere territories (as in Cbx^2, Cbx^{M1}) whether in symmetrical or asymmetrical (Cbx^3 and Cbx^{Twt}) transformations. The clonal origin of these separated rows of chaetae can be ascertained in these apogenetic mosaics by cell lineage analysis. Thus we have found that the clone includes one row or one row plus the haltere territory stripe extending to the other row (Figures 8 and 9). We interpret this behavior as (1) confirming that the D/V compartment boundary elicits an induction for chaetae formation (SANTAMARIA and GARCÍA-BELLIDO 1975; MORATA and GARCÍA-BELLIDO 1976), (2) a second signal appears at a given distance from the D/V boundary, defining the location of the opposite row of chaetae and (3) between these rows remains a territory of haltere trichomes that does not differentiate chaetae. We do not know whether or not this inter-row territory corresponds to an independent lineage that appears late in wing development and is obliterated later but is retained in the haltere. Late lineages for vein histotypes (such as the costa in which the triple row differentiates) have been found in normal developing wings (M. A. GONZÁLEZ-GAITÁN and A. GARCÍA-BELLIDO, unpublished data). Alternatively a second row of chaetae in haltere territory may correspond to a genuine, but hidden, extra row of chaetae that we encounter in other species of the genus *Drosophila* (GARCÍA-BELLIDO 1983).

We have seen in mosaic wing anlagen of *Cbx* alleles (and in *bx* and *pbx* halteres) in which one compartment (A or P) is histotypically transformed that the other compartment is affected in its size (Table 2). In the metathorax the smaller (transformed) wing size can be due to allele hypomorphism, but that cannot explain the increased size of the remnant haltere. In the mesothorax the haltere territory can also be larger than the corresponding wild-type haltere compartment, the wing territory being smaller than its normal counterpart. Here the larger haltere may be due to late transformation in a larger wing cell population. Reciprocally, the smaller wing could be explained by early transformation of a haltere growing population. In those cases of clonal *Cbx* this can hardly be the explanation. Possibly in both meso and metathoracic transformations involving compartment border cells, the A/P boundary moves, expanding the number of cells in the opposite compartment.

The problem of allele spatial specificity: Previous discussion indicates that the *Cbx* transformations result from complex cell responses. These include (1) cell heritability of early or late commitments and its eventual reversibility, in some alleles, (2) cell communication between adjacent cells leading to recruitment into either of two alternative pathways in other alleles and (3) short range accommodation between neighboring territories to match different cell proliferation dynamics and patterns (including D/V symmetries) in all *Cbx* alleles. We have to consider, in addition, that the presence in the genome of the *Ubl* mutation or of *Minute* mutations modify the pattern of expression of some *Cbx* alleles, possibly converting variable amounts of Ubx products into more or fewer cells (pattern extent) involved in the spatial transformation. Finally we should remember that the Cbx phenotypes differ in the hemizygous, heterozygous or homozygous condition, indicating again that amounts of *Ubx* gene derepression are transformed into spatial extent of *Ubx* gene expression. These considerations are crucial when considering the problem of the bases of allele spatial specificity.

There are certain correlations between type (clonality) of apogenetic mosaicism, the topographical pattern of the transformation and the *cis* perturbation in the molecular map of the *Ubx* gene (Figure 1). Thus both Cbx^3 and Cbx^{Twt}, affecting exclusively the A compartment and having clonal transformations, map in the 3′ end of the last Ubx transcription unit exon. Cbx^{M1} is not molecularly mapped but has a phenotype in homozygotes very similar to both Cbx^2 and *Hm* heterozygotes, the three possibly being either late clonal (Cbx^2, Cbx^{M1}) or not clonal. Cbx^1 (and probably its revertant Cbx^{1RM}) corresponds to an insertion of DNA from the pbx region in the first intron of the Ubx unit close to its promoter, and this mutation affects both A and P compartments. Transformed territories are not clonal in the P compartment but we cannot exclude clonality in the A compartment.

We do not know the nature of the *cis*-associated mutation in the two revertant alleles here studied. From these and previous considerations it is tempting to conclude that the specificities of the *Cbx* alleles are not dictated by the adjacent foreign DNA sequences in chromosome rearrangements, but are defined by the nature of their perturbations in the *Ubx* gene. With this in mind we can now group the *Cbx* alleles in two main classes: a first group, including Cbx^3 and Cbx^{Twt}, and a second one including the remaining alleles. We postulate that there is a positive feedback loop which maintains *Ubx* gene expression (CABRERA, BOTAS and GARCÍA-BELLIDO 1985; BIENZ and TREMML 1988; BOTAS, CABRERA and GARCÍA-BELLIDO 1988; MICOL and GARCÍA-BELLIDO 1988), and that *Ubx* expression is negatively controlled by repression in the anterior wing anlage. In Cbx^3 and Cbx^{Twt} the mutant modification of the *Ubx* gene structure (enhancer) or product (RNA) prevent this negative control from operating. In any case, the mutant condition would then be maintained in successive cell divisions by autoregulation, since only the autoregulatory positive signal remains. We must add that the A compartment spatial specificity may result from interference with *abx* and *bx* signals in this autoregulatory process (MICOL and GARCÍA-BELLIDO 1988; MICOL, CASTELLI-GAIR and GARCÍA-BELLIDO 1990). An alternative hypothesis (BUSTURIA *et al.* 1989) explains the mutant condition of these alleles from the deletion of a *cis*-negative control region exclusive to parasegment five. In the second group the mutational interference could be upon the mechanism of transcription control at the promoter region of the Ubx unit, thus affecting cells in both A and P compartments. This will allow for *trans*-reversible repression, following cell interactions.

As shown in Figure 3 the *Cbx* alleles show more subtle specificities within compartments. These specificities apply even to clonal *Cbx* alleles, reflecting internal heterogeneity of these regions during proliferation of the anlage (DIAZ-BENJUMEA, GONZÁLEZ-GAITÁN and GARCÍA-BELLIDO 1989). This heterogeneity may be inferred from the position of the A/P boundary where transformation stops very commonly in *Cbx* alleles that can affect both compartments (Cbx^1 and Cbx^2). We postulate that these heterogeneities convey the *trans*-signals involved in recruitment and accommodation (including D/V symmetries). It is interesting to mention in this context that the different *Cbx* alleles show *Ubx* gene derepression exclusively in the mesothoracic segment. But some of them (Cbx^3, Cbx^1 and *Hm*) can have UBX expression also in the head-antenna when this is transformed, by a variety of genetic backgrounds, into mesothorax (J. BOTAS and A. GARCÍA-BELLIDO unpublished results). This indicates that the *Ubx* gene of *Cbx* alleles is exposed to *trans*-controls defined by the mesothoracic (wing) developmental system upon which it itself operates.

These conclusions can be extended also to the mechanisms of morphogenetic control by *Ubx* gene in its normal realm of expression. We have only to assume that *Ubx* gene expression results from interactions between three components: (1) *Ubx* gene products involved in autoregulatory mechanisms, (2) products of unspecific *trans*-regulatory genes, such as *Polycomb* and *Regulator of Bithorax* (CAPDEVILA, BOTAS and GARCÍA-BELLIDO 1986, reviewed in DUNCAN 1987), and (3) products of genes which are involved in patterned cell proliferation and morphogenesis. The latter would provide for the fine tuning that leads, via cell interactions, to recruitment and accommodation in modes of cell proliferation and pattern formation (DIAZ-BENJUMEA, GONZÁLEZ-GAITÁN and GARCÍA-BELLIDO 1989). These interactions cause the modulation of *Ubx* gene expression in the cells of the growing haltere anlage, by the same morphogenetic genes that operate in the wing (including vein forming genes). These interactions can be altered by mutations in any of the three mechanisms, and in particular by the two main types of *cis* damage proposed for the *Cbx* alleles.

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